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Analysis and Design of a Photonic Biosensor for Mild Traumatic Brain Injury

by Mark A. Mentzer

ARL-TR-6372 **March 2013**

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14. ABSTRACT
This report describes a molecular biosensor that includes a lipid vesicle and housing wherein the vesicle is contained on or within the housing and where the housing has a portion capable of transmitting a force generated external to the housing to the vesicle. The biosensor detects the presence or absence of an event force, such as a blast or blunt force sufficient to produce a medical complication, such as traumatic brain injury.

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1. Introduction

The field of clinical neurology needs a detection system that can identify and quantify the extent of trauma an individual has received and allow for rapid-treatment decision making in the field or in a clinical setting. Secondary injury to a central nervous system tissue associated with the physiologic response to an initial insult resulting from direct blunt force or the percussive forces found in close proximity to a blast source could be lessened if the initial insult could be rapidly diagnosed or characterized. While the diagnosis of severe forms of such insults and the resulting damage is straightforward through clinical response testing, and computed tomography and magnetic resonance imaging testing, these diagnostics have their limitations. Medical imaging is both costly and time-consuming, while clinical response testing of incapacitated individuals is of limited value and often precludes a nuanced diagnosis. In many instances, the instrumentation necessary for these diagnostic procedures is not available in the field. Additionally, owing to the limitations of existing diagnostics, situations exist under which a subject experiences a stress to their neurological condition such that the subject often is unaware that damage has occurred or does not seek treatment as the subtle symptoms often quickly resolve. The lack of treatment of mild to moderate challenges to neurologic conditions can have a cumulative effect or subsequently result in a severe brain damage event that has a poor clinical prognosis.

Analyzing mechanisms and developing blast injury biomarkers are complicated by a deficiency of quality experimental studies and by the lack of sensitivity and specificity of biomarker-based injury prediction. Moreover, by the time a medic performs a biomarker analysis, the subject may be already in a severe and irreversible state of damage. Thus, the field of clinical neurology needs a detection system that can identify and quantify the extent of trauma an individual has received and allow for rapid-treatment decision making in the field or in a clinical setting.

2. Summary of Proposed Sensor Construct

A molecular biosensor would be useful in the laboratory or in the field for detecting a blunt force or a blast force event. Such events often cause traumatic brain injuries. With traumatic brain injuries, and particularly with mild traumatic brain injuries, there may be no external signs of injury, which could delay treatment or indicate that no treatment is necessary. This can lead to severe and often cumulative consequences. The molecular biosensor includes a lipid vesicle (figure 1) on or within a housing that will not appreciably alter the event force transmitted to the vesicle. A lipid vesicle may be tailored to include one or more lipids and optionally other molecules, including proteins and cholesterol, to serve as a model similar to the plasma membrane of brain tissue.

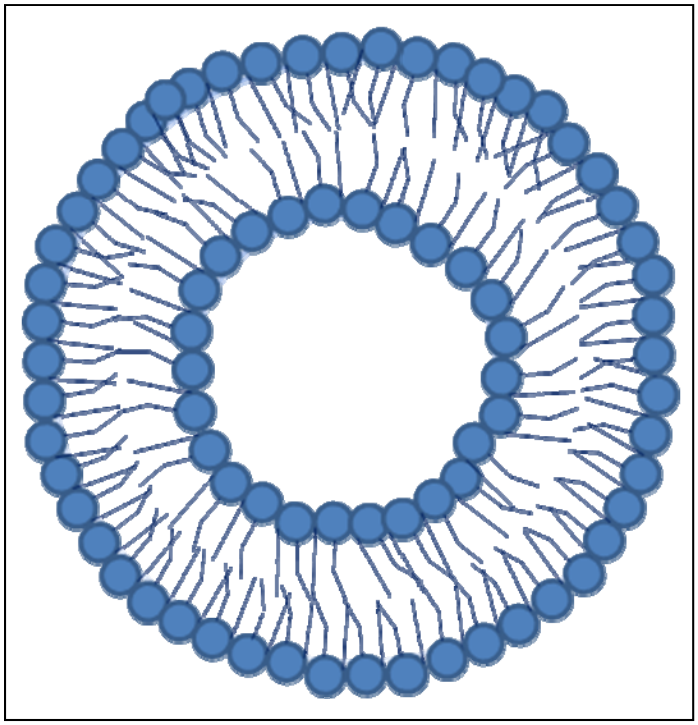

Figure 1. Conceptual diagram of self-assembled liposome construct, illustrating hydrophobic orientation of acyl tails and hydrophilic head groups

Illustrative components of a lipid vesicle in the biosensor include phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, phosphatidylinositol, sphingomyelin, cholesterol, ceramide, and one or more proteins, or combinations thereof. The molecular biosensor is useful for the detection, diagnosis, or study of a traumatic event, such as blunt force, blast force, or other force of sufficient magnitude to produce a traumatic brain injury in an animal or human subject. By affixing a molecular biosensor to an article of protective clothing or other location on a Soldier, athlete, or other subject, medics can readily ascertain the magnitude of an event. This information could be used to direct the wearer to medical attention or for the study of the ability of protective articles to protect a wearer. We thus address the need for a biologically relevant correlate to traumatic injuries that can be used in either a field or laboratory setting.

3. Detailed Description of the Sensor

A significant technology gap exists in testing personal protective equipment for the military, in the area of body armor and helmet systems. Sensors are required to determine the correlation between threats (insults) to the Soldier to provide a means by which protective equipment can be assessed for its ability to protect a Soldier from a variety of insults, and to optimize the design trades between armor weight, thickness, energy dissipation, and stopping power. This need

extends to the widely publicized concerns regarding protection of Soldiers in battle and to the protective measures needed for contact sports, especially American football.

A recent understanding of the medical condition known as traumatic brain injury (TBI), chronic traumatic encephalopathy, certain aspects of posttraumatic stress disorders, and their associated symptoms further illuminates the need for improved understanding of the effects of severe trauma to the head or torso. Improved prophylaxis includes armor designed to better shield from the insult scenarios, as well as improved postexposure treatment to alleviate or minimize the short- and longer-term effects of the insults. A gamut of intracranial pathologies results in symptoms including loss of memory, disorientation, angiogenesis, and long-term cognitive disorders.

We require a means to measure the range of insults, including blunt trauma, ballistic impact (often collectively referred to as B&B), and shock trauma, with a metric that directly indicates the injury to the body due to an insult, thereby directly correlating insult to injury. A host of sensors has been employed to this end—including pressure sensors, accelerometers, strain sensors, and optical surface measurement methodologies. These sensors characterize the energy impacting the protective armor and the dissipation of that energy through human tissues. A range of torso and head form anthropomorphic test modules (ATMs) incorporating these sensors, the point and two-dimensional (2-D) energy characterizations, along with time-resolved networked sensor determinations, have all provided less than satisfactory correlation of insult to injury.

While many candidate sensors continue to emerge in the literature (hydro gels, functionalized nanoparticles, photonic crystals, etc.), only the novel sensor concept described herein directly represents the response of human tissues to traumatic insult. Nanotechnology research is replete with examples of self-assembled chemicals forming well-controlled supramolecular films and structures, including manipulation of material properties at the atomic level of detail.

The issues with current sensors used in test labs, as well as those included in ATM's and even athletes' helmets, include lack of repeatable measurement, poor to no correlation, lack of calibrated response to the range of insults to include ballistic threats, and concurrently, lack of correlation to any or all of the range of tissue susceptibilities and widely varying vulnerabilities. Test artifacts abound because of a wide range of variables, including threat mass, velocity, total yaw at impact, yaw cycle precession, obliquity at impact, backing material variability, along with backing material inconsistencies, tissue simulant variation, and lack of controlled test protocols proving repeatability of test metrics. These variables result in highly conservative limits for penetration depth at prescribed impact kinetic energies, providing only partially correlated determination of armor suitability and little trade space for the armor designer to effect improvements.

Bullets and fragments cause tissue injury a number of ways, even if the impact is nonpenetrating. The amount of kinetic energy transferred to and through the tissues correlates to the severity of

the tissue damage, which is determined by four key factors.[1] These include kinetic energy (1/2 mv^2) at impact, total yaw at impact, shape of the insult, and the characteristics of the target tissue (density, strength, and elasticity). Nonpenetrating events causing tissue damage mechanisms may be collectively addressed as the disruption of the phospholipid bilayer surrounding the cellular structure of human tissues. Relative damage to tissues correlates directly to tissue densities such that a measure of lipid bilayer disruption by the threat provides a very direct and novel approach to the lingering problem of insult-to-injury correlation.

The drawbacks of prior sensor systems and processes of their use are addressed by physiologically relevant sensors. Such sensors can be used to test the ability of protective equipment to protect an individual's brain or other organs from certain traumatic events. Thus, a device is provided that can indicate whether a traumatic event may have caused a traumatic brain injury and as a sensor that can determine the ability of protective equipment to protect against particular threats. The sensor described has utility as a detector and as a method of detecting the presence or absence of an event sufficient to produce a traumatic brain injury, such as mild traumatic brain injury (mTBI).

The sensor uses self-assembled liposome structures to detect an event sufficient to cause mTBI or TBI. The sensor is configured and packaged in a manner where the sensors can be affixed to Soldier helmets and body armor personal protective equipment and provide a direct indication of the trauma received at the point of attachment. This trauma indicator relates and is equivalent to the blunt and ballistic trauma, as well as the convolved effect of shock waves associated with blast trauma, received by the human body tissues during equivalent events. The sensor, therefore, represents the first real and direct measure by which insult is correlated to injury. The disruption of the phospholipid bilayer occurring to a human or other subject representing damage to human tissue is the very same disruption measured by the sensor. Thus, in correct embodiments, this sensor may provide a more accurate measure of damage to brain tissue caused by a blast.

The molecular biosensor system includes a lipid vesicle and housing. The lipid vesicle may be contained within the housing or otherwise attached to or retained by the housing. An event such as a shock wave or blunt force when contacting the housing is transmitted to the lipid vesicle, altering a molecular characteristic of the vesicle that correlates with and indicates the degree, type, duration, severity, or other characteristic of the event force. The lipid vesicle is sufficiently related to the plasma membrane of a brain cell by possessing similar lipid and, optionally protein content, to the plasma membrane of a brain cell such that molecular alterations in the vesicle correlate to damage of a neuron when exposed to an event force.

[1]Cooper, G. J.; Ryan, J. M. Interaction of Penetrating Missiles With Tissues: Some Common Misapprehensions and Implications for Wound Management. *Br. J. Surg.* **1990**, *77*, 606–610.

Vesicles that are sufficiently related likely possess a majority lipid composition of phosphatidylcholine (PC) and/or phosphatidylethanolamine (PE). The molecular biosensor will likely include lipid vesicles that are predominantly PC, PE, or have a lipid content that is a 50% or greater combination of PC and PE. Illustrative examples of the lipid content of the plasma membrane are found in the literature.[2] In successful embodiments, the amount of lipid (e.g., phospholipid, sphingomyelin, and cholesterol) is likely represented by the amounts presented by Scandroglio et al.[2]

A lipid vesicle may also be formed from PC, PE, phosphatidylserine (PS), phosphatidylinositol (PI), sphingomyelin, cholesterol, ceramide, or combinations thereof. In some embodiments, a lipid vesicle is substantially PC or a combination of PC and PE. Lipid vesicles optionally contain only PC as a lipid component. In some embodiments, the concentration of PC is from 30% to 80% of total lipid weight. A lipid vesicle optionally includes PS. When present, PS is found at from 5% to 30% of total lipid weight or any value or range there between. In some embodiments, PS is present at 5%, 10%, 15%, 20%, 25%, or 30% of total lipid weight.

In other embodiments, a lipid vesicle includes sphingomyelin. Sphingomyelin is optionally present at from 5% to 30% of total lipid weight, or any value or range there between. In some embodiments, sphingomyelin is present at 5%, 10%, 15%, 20%, 25%, or 30% of total lipid weight. Some embodiments include PC/PE/PS combinations where the PC/PE is present in a majority by total lipid weight. An illustrative example includes PC and PE present at amounts that total 50% up to substantially 100% by total lipid weight. Sphingomyelin is optionally present at a lipid percentage of 1%–30% total lipid content, or any value or range there between. In some embodiments, sphingomyelin is present at 1%, 5%, 10%, 15%, 20%, 25%, or 30%. Cholesterol is optionally present at an amount relative to the lipid portions of 0.5 cholesterol/ lipid or less. In some embodiments, the amount of cholesterol is present at a ratio of 0.01 to 0.5 cholesterol/lipid or any value or range there between. Optionally, the amount of cholesterol is present at a ratio of 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, or 0.5 cholesterol/lipid.

Lipid vesicles may contain other materials, such as proteins or fragments of proteins, that may or may not alter the fluidity of the membrane or provide a membrane with a protein content similar to that of a brain neuron as is known in the art. While the lipid vesicles described are provided as examples, lipid vesicles can include one or more of PC, PE, PS, PI, cholesterol, ceramide, sphingomyelin, and protein in any combination, and all combinations are appreciated as envisioned under the invention. In some embodiments, a lipid vesicle includes PC alone to the exclusion of other lipids, cholesterol, or protein.

[2]Scandroglio, F.; Venkata, J. K.; Loberto, N.; Prioni, S.;, Schuchman, E. H.;, Chigorno, V.; Prinetti, A.;, Sonnino, S. Lipid Content of Brain, Brain Membrane Lipid Domains, and Neurons From Acid Sphingomyelinase Deficient Mice. *J. Neurochem.* **2008**, *107* (2), 329–338.

4. Vesicle Formation

Methods of forming a lipid vesicle are well known in the art and are applicable to the formation of lipid vesicles as a portion of a biosensor. For example, lipid vesicles formed by techniques for assembling the liposome structures are well characterized, since liposomes are the basis for several novel drug delivery systems and are therefore well developed. The basic process involves hydration of dry lipid, cholesterol, protein, or other component of the lipid membrane onto a vessel surface from organic solvent (e.g., chloroform), thereby producing a thin film of dry lipid. This material is then hydrated to solution typically in an aqueous buffer system such as Tris-buffered saline, HEPES-buffered saline, water, or other suitable buffer and forming the liposomes as the solution is heated above the liposome phase transition. As many lipids have a phase transition that is below room temperature, heating is not always necessary, depending on the total composition of the lipid membrane.

Concentric lipid bilayers result in the form of controlled 30- to 70-nm-diameter liposome spheres. Freeze-thaw processing further refines the liposome morphology. As such, in some embodiments, lipid vesicles are formed by sonication of the hydrated material, typically on ice to prevent overheating, or by one or more freeze-thaw cycles. The resulting liposomes are optionally sized by chromatography or passing through one or more filters of desired pore size.

5. Incorporation of Detection Agents

In some embodiments, a lipid vesicle includes one or more detection agents. A detection agent is any molecule that is encapsulated by a lipid vesicle that can be released upon vesicle rupture and thereby detected. Illustrative examples of detection agents include dyes, fluorophores, nucleic acids, proteins, combinations thereof, or other detection agents. By encapsulating one or more detection agents in the liposome (either in the space within the liposome or the lipid monolayer, bilayer, or multilayer) during the self-assembly process, a lipid vesicle is provided that allows a detectable color or other change from the trauma-induced liposome disruption that is proportional to the amount of disruption or insult.

This process affords a very attractive additional feature, whereby a change in color indicates that a more precise measurement should be taken at the Soldier clinic (or athletic field or arena sideline) as the basis for determining prophylaxes and posttrauma expectations. Epidemiological data can also be accumulated rapidly to assess various treatment options to save lives and minimize postexposure conditions.

Illustrative examples of detection agents include fluorophores such as calcein, pyranine (1-hydroxypyrene-3,6,8-trisulfonic acid), and FAM dye (illustratively 6-carboxyfluorescein). Other fluorophores illustratively include TAMRA, AlexaFluor dyes such as AlexaFluor 495 or 590, Cascade Blue, Marina Blue, Pacific Blue, Oregon Green, Rhodamine, Fluoroscein, TET, HEX, Cy5, Cy3, Quasar670, and Tetramethylrhodamine. Specific examples of fluorophores include 5-(and-6)-carboxyfluorescein mixed isomers, Alexa Fluor 647 carboxylic acid, and succinimidyl ester (Alexa 647).*

Other illustrative examples of a detection agent include the pH-sensitive dyes. An illustrative example of a pH-sensitive dye is acridine orange. A detection agent is optionally a nucleic acid–sensitive agent. An illustrative example of a nucleic acid sensitive agent is Hoechst 33342 (2,5'-Bi-1H-benzimidazole, 2'-(4-ethoxyphenyl)-5-(4-methyl-1-piperazinyl)). The casein dyes are similarly nucleic acid sensitive. In some embodiments, a detection agent is bound to a retention molecule to prevent leakage from the lipid vesicle prior to rupture. Such retention agents are any membrane-inert material, such as biotin, polyethylene glycol, antibodies, or other material known in the art.

In another approach, the detection agent is nucleic acid sensitive. A nucleic acid is optionally bound to a surface of a housing such as by reaction with a polystyrene plate. A nucleic acid molecule may be aminated to promote binding to a polystyrene section of a housing via the amine bond.† When a nucleic-sensitive dye such as 4', 6-diamidino-2-phenylindole, or the cell impermeant green nucleic acid stain is released upon lipid vesicle rupture, it will bind a nucleic acid molecule on the surface of the housing, localizing colorimetric detection of lipid vesicle rupture.

In some embodiments, a detection agent is a nucleic acid, antibody, protein, or other molecule suitable for specific interaction with a binding partner. Illustratively, the nucleic acid used is suitable as a primer for a polymerase chain reaction. Extraction of a portion of the extra liposomal solution and its inclusion in a polymerase chain reaction will positively or negatively discern whether the nucleic acid–based molecule has leaked from the lipid vesicle. Other detection agents include antibodies that can specifically bind to an antigen bound to a surface of a housing. The housing surface can then be probed by a process similar to an enzyme-linked immunosorbent assay to detect the presence or absence of the antibody; other detection agents are similarly suitable.

Freeze-thaw processing during lipid vesicle formation (e.g., two to three cycles) further refines the liposome morphology and encapsulates one or more detection agents from a biphasic mixture for colorimetric sensor features. Note that certain fluorophores are activated when exposed to solvent reagents and/or water, thereby effecting the color change for direct insult observation.

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*Each available from Life Technologies, Grand Island, NY. Other fluorophores are similarly suitable.

†Illustrative technology is available from Corning, Inc., Tewksbury, MA, sold as the DNA-BIND polystyrene material.

As such, in some embodiments, one or more fluorophores are encapsulated in a lipid vesicle formed in a nonaqueous solution. The resulting vesicles are then washed and placed in an aqueous medium for association with a housing. Upon rupture due to an event force, the detection agent escapes the lipid vesicle and is detectable by a direct color change. This color change allows the medic to quickly and easily identify a situation requiring medical attention.

The liposome-based sensor can be incorporated in a variety of anthropomorphic test modules and instrumented head forms (collectively housing) currently available, supplanting or at least supplementing the less effective pressure sensors and accelerometers that previously provided far less than optimal correlation to mTBI or TBI at best. As the applications are validated for the sensor, a variety of housing options are available; these include lamellar encapsulation of the liposomes themselves, incorporation in solution, and containment in honeycomb-interlaced sheets of material as well as tablets or ampoules for convenient attachment at vulnerability points of interest. Sensor arrays can also be implemented to provide dimensionality and spatial control of the event characterization. Placing the sensor as close as possible to the vulnerable areas of concern thus provides a direct and more accurate measure of the tissue susceptibility to a threat combination or singular test scenario.

A housing is either a surface, whereby the lipid vesicles are exposed directly to the environment, or the housing encapsulates the lipid vesicles, whereby the housing has at least one surface that will transmit an event force from the external environment to the lipid vesicle. Optionally, at least one portion of a housing is transparent to a wavelength of light emitted or reflected by a detection agent. In some embodiments, a housing is in a capsule form. Optionally, a housing is in a cubic or rectangular prism form. Or, a housing is in a spherical, sheet, curvilinear, or other two- or three-dimensional shape. One example of a suitable housing is similar to a dialysis cassette sold as a SLIDE-A-LYZER, available from Thermo Fisher Scientific, Inc., Rockford, IL. Such a housing has a membrane on one or both sides that will transmit an event force to a lipid vesicle contained within the housing. Such embodiments can also encapsulate one or more detection agents into the vesicle and then transfer buffer or wash away any excess dye after vesicle formation simply by buffer exchange right in the housing.

Optionally, a housing is in the form of a capsule. Capsules can be formed of any material traditionally known in the art that will transmit an event force to a lipid vesicle. Illustrative materials include gelatin, starch, casein, chitosan, soya bean protein, safflower protein, alginates, gellan gum, carrageenan, xanthan gum, phtalated gelatin, succinated gelatin, cellulosephtalate-acetate, oleoresin, polyvinylacetate, hydroxypropyl methyl cellulose, polymerisates of acrylic or methacrylic esters, polyvinylacetate-phtalate, and combinations thereof. An illustrative capsule is described in U.S. Patent application 2005/0008690.[3]

[3]Miller, F. H. Multi-phase, Multi-compartment Capsular Delivery Apparatus and Methods For Using Same. U.S. Patent application 0008690 A1, January 13, 2005.

A housing is optionally formed in whole or in part of polymeric materials. Illustrative examples include flexible vinyls (e.g., polyvinylchloride), polyamides, polypropylene, norell, polysulfone, ABS, polyethylene, natural and synthetic rubbers, among many others.

The molecular biosensors provided have the capability to detect, measure, quantify, and optionally correlate an event force to the likelihood or severity of TBI or mTBI suffered by a wearer of the biosensor. As such, processes of detecting and, in some embodiments, quantifying blast or other direct event force using self-assembled liposome structures as a unique sensor are provided. The liposome structures are configured and packaged in a manner where the sensors can be affixed to Soldier helmets and body armor personal protective equipment in a manner that directly indicates the trauma received at the point of attachment. As such, in some embodiments, a molecular biosensor is affixed to an item of clothing, such as a helmet or other protective equipment, or to a traditional clothing form. A molecular biosensor is optionally affixed to a building or vehicle surface. A molecular biosensor has utility for the detection of blunt and ballistic trauma, as well as the convolved effect of shock waves associated with blast trauma, received by the human body tissues during equivalent events. The sensors provided represent the first real and direct measure by which insult is correlated to injury. The disruption of the phospholipid bilayer in human tissue resulting from such forces is directly measured by the liposome sensors in the most meaningful way possible—by the identical disruption that occurs to a human subject.

A process of detecting a traumatic event is provided, including subjecting a molecular biosensor to an event force and analyzing the biosensor or lipid vesicle for alteration indicative of an event force sufficient to produce TBI or mTBI. As used herein, an "event force" is any force type suitable to produce or model a traumatic brain injury of any form. Such forces include, but are not limited to, blunt force, ballistic force, and shock wave forces (illustratively those associated with blast trauma). A shock wave generator is one possible source of an event force. Other shock wave generators are capable of producing a shock wave event force. A blunt force trauma is optionally produced by any source of such force. Experimentally, blunt force events are produced by fluid percussion, cortical impact, and weight drop/impact acceleration sources.* A molecular biosensor is used in a process of detecting a traumatic event, or a lipid vesicle absent a housing is used. The presence or absence of a traumatic event is determined by an alteration in the lipid vesicle itself or by an alteration in the amount, type, binding, or other characteristic of a detection agent present in the lipid vesicle or on the lipid vesicle.

In some embodiments, a traumatic event is determined by an alteration in the molecular structure or orientation of one or more molecules that make up a lipid vesicle. Illustratively, circular dichroism is used to detect molecular alterations in one or more components of a lipid vesicle. The sample material to be analyzed is contained in a quartz cylinder, within which are spacers to

*See e.g., Alder, J. et al. *J Vis Exp* **2011**, Aug 22 (54) and Cernak, I. Animal Models of Head Trauma. *NeuroRx* **2005**, *2*, 410–422.

accommodate smaller sample vessels. An alternate sample contained may be a Hellma Analytics photometric micro tray cell cap. Alternate packaging schemes may be developed to provide insult maps across curved and rectilinear tessellations for certain applications. Linearly polarized light is passed through the analyte. In a chiral material such as the liposome, the right and left circularly polarized components travel at different velocities and are differentially absorbed. This results in the light exiting the analyte with elliptical polarization, and the analyte is deemed to possess circular dichroism (CD). The magnitude of CD is expressed as the molecular ellipticity θ:

$$\Theta = 4500/\pi\ (\epsilon_L - \epsilon_R)\log_c 10\ , \tag{1}$$

where ϵ_L and ϵ_R represent the molecular extinction coefficients for the right and left circularly polarized light beam components. The difference between the extinction coefficients is

$$\Delta\epsilon = (\epsilon_L - \epsilon_R) = 1/LC\ \log_{10}(I_R/I_L)\ , \tag{2}$$

where L is the absorbing layer thickness (cm), C is the molar concentration, and I_R and I_L are the intensities of the right and left circularly polarized light beams after passing through the analyte. θ then becomes

$$\Theta = 4500/\pi LC\ \ln 10 \log_{10}(I_R/I_L)\ . \tag{3}$$

Circular dichroism spectrometers* measure CD changes of the magnitude produced in the sensor. Differential CD is created by disruption of the intramolecular chiral interactions of three-dimensional molecular structures, as well as the additional chiral symmetry breaking of nonchiral molecules in the sensor material construction. Measurement in changes of CD are indicative of alterations in the structure of the lipid vesicle and indicative of an event force.

A traumatic event is detected by analysis of a medium external to a lipid vesicle. For example, in some embodiments, a lipid vesicle includes one or more detection agents within the vesicle. The leakage of a detection agent(s) into the extra liposomal space is indicative of damage to the lipid membrane, such as rupture or more minor damage. Optionally, a detection agent is a fluorophore. Or, the fluorescence is quenched because of high dye concentration internal to the liposome and unquenched upon release of the dye molecules into the surrounding solvent upon event force. Instrumented techniques based on atomic force microscopy, confocal fluorescence microscopy, and fluorescence recovery after photo bleaching optionally coupled with colorimetric fluorometry (detection of light intensity based on leakage of dye from the disrupted cell wall) are used to analyze liposome disruption and failure criteria. The rate of detection agent release is optionally accomplished through cholesterol-influenced bilayer properties where higher levels of cholesterol typically equate to a less fluid and more event force–resistant membrane.

*Such as the J-815 from Jasco Corporation, Easton, MD.

The molecular biosensors and processes described are useful in many areas:

- In military applications for the design of protective equipment, such as body armor and helmets.
- For rapid diagnosis or prediction of possible TBI or mTBI in a subject receiving an event force in the field to provide or indicate the need for medical intervention.
- As a point sensor or array of sensors to provide 2-D mapping of trauma.
- As a sensor useful in the design and use of sport-related protective headgear in which concussions and other brain injuries are a concern.
- As a sensor for research, testing, and/or development of protective equipment by athletic equipment manufacturers and military equipment manufacturers.
- As a research tool to understand the molecular results of forces that produce traumatic events and complications such as TBI or mTBI.

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